SCWDS BRIEFS

A Quarterly Newsletter from the

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Volume 33 **July 2017** **Number 2**

60 Years of One Health Work at SCWDS

July 1, 2017, marked the 60th anniversary of the Southeastern Cooperative Wildlife Disease Study (SCWDS). On July 1, 1957, the Southeastern Association of Game and Fish Commissioners (SEAGFC, now known as the Southeastern Association of Fish and Wildlife Agencies) founded the Southeastern Cooperative Deer Disease Study (SCDDS). Headquartered at the University of Georgia's College of Veterinary Medicine and directed by Dr. Frank A. Hayes, the SCDDS mission was to investigate the recurring mysterious deer mortality events (which turned out to be hemorrhagic disease) that threatened extensive deer restoration efforts following World War II. Eleven southeastern state wildlife management agencies were founding members of the cooperative. Member states later grew to 13, then to 15, and currently number 19. State members are the wildlife resource agencies of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Virginia, and West Virginia. Additional supporters today include APHIS-Veterinary Services and -Wildlife Services of the U.S. Department of Agriculture and the U.S. Geological Survey and the U.S. Fish and Wildlife Service of the Department of the Interior.

Shortly after the inception of SCDDS, those involved with it became aware of the lack of information on white-tailed deer diseases. In addition to requests to develop data on deer diseases, there was increasing pressure on SCDDS' small staff to procure vital information on potential disease interrelationships between wild animals, humans, and domestic animals. Recognizing the increasing demands on the newly created SCDDS, in 1960 SEAGFC expanded the mission to encompass all wildlife species and changed the name to the Southeastern Cooperative Wildlife Disease Study (SCWDS), as we are known today. Through the efforts of several southeastern states, in 1963 the U.S. Congress enacted a recurring annual appropriation, administered through the Department of the Interior, to support wildlife disease research ats SCWDS. Through these means and additional support from other funding agencies and collaborators, efforts began to close information gaps about diseases in wild animals and disease interactions between wildlife, domestic animals, and humans.

Understanding and addressing disease interactions between humans, animals, and the environment are best achieved through a One Health approach. According to the World Organization for Animal Health (OIE), the One Health concept "*summarized an idea known for more than a century that human health and animal health are interdependent and bound to the health of the ecosystems where they exist*."

"One Health" put a name on 60 years of work SCWDS has been doing to better understand diseases within the triad of human, animal, and environmental health. We want to take the occasion of our 60th anniversary to highlight some of the SCWDS contributions to One Health. The following articles cover selected One Health-oriented projects and nearly all of them were collaborative with scientists in fields other than wildlife health. We regret that space does not allow us to include more, such as our development of the bait now used to orally vaccinate wild animals for rabies, wildlife surveillance for zoonotic pathogens like *Brucella* spp., and tracking the emergence of a poultry pathogen (*Mycoplasma gallisepticum*) in an invasive avian species in the eastern U.S.

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Sharing the Flu

Virtually all of us know about influenza from personal experience. Influenza A viruses (IAV) are among the most important human pathogens, occur annually across the globe, and represent one of our most feared pandemic threats. These viruses also can cause devastating losses to poultry, are a recurring problem in domestic swine, and even infect domestic horses, dogs, and cats. We have known for a long time that wild birds, especially waterfowl, shorebirds, and gulls, are the historic reservoirs for these viruses, and there has been a great deal of research and surveillance directed at these populations. What do we gain from all of this, and why is such work more important than ever? To understand this, it helps to look at how our knowledge of influenza in these wild bird populations has evolved. Although this evolution represents the combined work of numerous scientists worldwide, SCWDS researchers have been and continue to be major contributors to this story.

Influenza research and surveillance related to wild birds started with discovery of these viruses in wild birds during the 1960's and 1970's. During the next three decades, we learned much of what we now know about avian influenza epidemiology, the specific wild bird species that comprise the reservoirs, the risk of spillover to domestic poultry populations, and the diversity of viruses maintained in these reservoirs.

Our work with influenza started in 1983 when SCWDS provided wildlife surveillance in support of highly pathogenic H5N2 eradication in poultry in Pennsylvania and Virginia. More recent work has been funded by USDA, CDC, and since 2007, we have been part of the NIH Centers of Excellence for Influenza Research and Surveillance. Through this support and resulting collaborations, we have contributed to further understanding the stability of these viruses and the potential for environmental transmission, seasonality of IAV subtype diversity in waterfowl populations, susceptibility of North American waterfowl species to highly pathogenic Eurasian H5 viruses, potential role of passerine species in IAV epidemiology, epidemiology of IAV in shorebird populations, and the potential role of population immunity in regulating IAV prevalence and subtype diversity in wild bird populations.

Challenges still exist. In the late 1990s, a new and more complicated story emerged that involved four unexpected events: 1) human disease and death associated with an avian virus of the A/Goose/Guangdong/1/1996 lineage of highly pathogenic H5 IAV; 2) an outbreak of this HPAI H5 virus in a Hong Kong zoo that infected and caused disease in a diversity of wild bird species including waterfowl; 3) the continued detection of these viruses and related mortality in wild birds in Asia; and 4) the continental spread of Asian HPAI H5 to Europe and Africa via wild bird migration that began in 2005 and resulted in high mortality among waterfowl and poultry. These and subsequent events, such as the spread of these viruses to North American wild birds and poultry during 2014, dramatically changed the wild bird story and created many new questions, such as, if and how these "new viruses" could be maintained in wild bird reservoirs.

The epidemiology of influenza always has connected wild bird, domestic poultry, and human health, but this story is changing and growing more important. The emergence and spread of Asian H5 viruses appears to be just the beginning. More and more poultry-adapted strains are being detected that are capable of infecting humans and causing severe disease in poultry. In addition, these viruses have the potential to spill over into wild bird populations resulting in disease and possible global spread.

We have come a long way in our understanding of IAV in wild bird populations since the 1960's. However, these are very complex systems, they involve numerous wild bird species and diverse virus populations, and many questions remain. What has become clear is that future success related to research, prevention, or control requires a One Health approach. On the research side, the questions asked by scientists involved in human, domestic animal, and wildlife health are often the same and often can be answered with the same tools and methodologies. On the prevention and control sides, wildlife, domestic animal, and human health are becoming more and more linked as contact increases between humans, poultry, and waterfowl. We must combine our collective expertise and dedication related to the health of humans, animals, and the environment if we are to adequately address such complex problems. (Prepared by David Stallknecht, Rebecca Poulson, and Charlie Bahnson)

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Ticks and Tick-borne Pathogens

SCWDS has worked with ticks and tick-borne pathogens for decades. Ticks are the second most important vector of human pathogens, behind mosquitos, in regards to the number and virulence of pathogens transmitted. Ticks also are important vectors of pathogens among domestic and wild animals, and many of these pathogens are of economic importance to animal agriculture.

Our initial work on zoonotic pathogens and ticks began in the early 1990s with *Borrelia* and expanded greatly later in the 1990s when Dr. Randy Davidson obtained National Institutes of Health (NIH) funding to support tick work at SCWDS. The early years were spent investigating the role of wildlife species, such as white-tailed deer and raccoons, as reservoirs of human pathogens including *Ehrlichia chaffeensis*, *E. ewingii*, *Ehrlichia* sp. PME, and *Anaplasma phagocytophilum*. These studies also aimed to better understand the vectors, and many host-tick associations were confirmed through a combination of field, experimental, and laboratory studies that were continued with additional support from NIH and the Southeast Center for Emerging Biologic Threats (SCBET)

In addition to the work on these zoonotic pathogens, several of which also are important for domestic animals, we have obtained important data on other veterinary pathogens such as *Cytauxzoon felis*, a nearly-always fatal parasite of domestic cats, *Hepatozoon* spp., pathogens of dogs, and *Babesia* spp. of various hosts. Novel species detected or described include *Anaplasma odocoilei* of white-tailed deer, *Babesia uriae* from common murres, '*Candidatus* Neoehrlichia lotori' from raccoons, a *Babesia* sp. of Florida pumas, and at least two *Babesia* spp. in raccoons. These studies were supported by sources including the Morris Animal Foundation, Sigma Xi, and SCBET.

Over the years we have worked with numerous local, state, and federal public health agencies to better understand the risk of tick-borne disease in humans. We have tested ticks for selected pathogens; conducted epidemiologic studies to determine behaviors that put people at risk for tick-borne pathogens; genetically characterized pathogens from people, animals, and ticks; and investigated the role of habitat management techniques, such as prescribed fire, on tick populations. More recently, we have developed collaborations with the Companion Animal Parasite Council (www.capcvet.org) with an emphasis on developing maps of the distribution of several zoonotic tick-borne pathogens. These data are used to model the distribution and forecast the risk of tick-borne pathogens in the upcoming months or year.

Much of our work is focused in North America, but we also have worked on ticks and tick-borne pathogens in Botswana, Brazil, Costa Rica, Grenada, Panama, South Africa, and Zambia, where we work to understand the risk these pathogens pose to wildlife, domestic animals, and people. (Prepared by Michael Yabsley)

Tropical Bont Ticks in the Caribbean

The tropical bont tick (TBT, *Amblyomma variegatum*) is native to Africa and was introduced to the Caribbean region on cattle brought from West Africa in the late 1700s to early 1800s. Since then, it has been found on islands from Barbados to Puerto Rico. In the U.S., the tick was declared eradicated in 1970 from St. Croix in the Virgin Islands Territory and in 1987 from the Commonwealth of Puerto Rico. However, the TBT was found again in 2000 in St. Croix and periodically thereafter, but has not been seen there since 2014.

The TBT is a vector of three important bacterial pathogens. *Ehrlichia ruminantium* is the etiologic agent of heartwater, a disease of domestic and wild ruminants that can cause high mortality rates. *Ehrlichia ruminantium* also was introduced from Africa and is present in domestic livestock in Antigua, Guadeloupe, and Marie Galante. The TBT also is associated with the exudative skin disease acute bovine dermatophilosis, which is caused by *Dermatophilus congolensis* and is capable of killing cattle in tropical climates such as the Caribbean. The TBT also transmits the human pathogen *Rickettsia africae*, which causes African tick bite fever, a disease characterized by skin lesions, fatigue, fever, and chills. This disease agent occurs in sub-Saharan Africa, is a common cause of imported fever among travelers returning from endemic areas in Africa, and is widespread among islands in the Caribbean region. *Rickettsia africae* is the most widely distributed of all spotted fever group rickettsiae that cause disease in humans.

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Heartwater is classified as a foreign animal disease by the USDA and is a threat to domestic cattle, sheep, and goats. However, wildlife implications also are significant: Experimental infections of white-tailed deer conducted at USDA's Plum Island resulted in mortality of 7/9 inoculated deer; one deer was euthanatized due to physical injury and another was treated successfully with tetracycline.

In Africa, wildlife hosts of the TBT include a wide range of mammals and birds. In the Caribbean, SCWDS and others have found larvae and nymphs on black rats, house mice, small Asian mongooses, black-faced grassquits, common ground doves, and cattle egrets. Infestation of wildlife may hinder control or eradication efforts in the Caribbean because wild animals may serve as maintenance hosts or disseminators of TBT on an island or between islands.

SCWDS has been involved in TBT eradication programs since 1985, and we have conducted surveys for wildlife hosts in Antigua, Guadeloupe, Puerto Rico, and St. Croix from 1988 to the present. We also conducted a Caribbean-wide study on movement patterns of cattle egrets to see if they were involved in spreading the tick between the islands. From 1988-91, SCWDS conducted surveys for TBT on cattle egrets in Antigua and Guadeloupe, and banded and marked 1,129 cattle egrets with colored dyes. Up to 10% of cattle egrets we examined were infested by the TBT. We received reports of sightings of marked birds on 14 islands in the Caribbean region and in the Florida Keys. Egrets moved regularly among the Caribbean islands, migrated to the United States, and may be responsible for at least some of the spread of the TBT within the Caribbean region. Fortunately, the tick has not been detected outside the Caribbean region in the Western Hemisphere. Either the ticks dropped from hosts before reaching more distant areas, such as the U.S. mainland, and/or introductions have not resulted in successful establishment of new populations.

The TBT infests a wide range of animals and is a vector of diseases that affect humans, wildlife, and domestic animals. This tick remains a significant One Health challenge in Africa and the Caribbean region, and would present additional health challenges if it were to become established in the United States. (Prepared by Joe Corn)

West Nile Virus and Wild Birds

West Nile virus (WNv) quickly became established in North America after its introduction to New York City in 1999. Within three years, the virus distribution had expanded from a 6-county area around metropolitan New York City to the West Coast and included 44 states and the District of Columbia, as well as five Canadian provinces. Today, WNv continues to cause morbidity and mortality in wildlife, humans, and domestic animals.

West Nile virus initially was detected in the U.S. during investigations of atypical mortality observed in American Crows in New York City. Wild birds, especially corvids (crows and jays), became sentinels for WNv activity and were used to track its spread and provide early warnings of public health risks. Most WNv surveillance and research projects at SCWDS have been associated with one or both of the objectives.

SCWDS began work on WNv in 2000 and continues today in surveillance and research efforts. Initially, we provided laboratory support to the Georgia Department of Public Health's WNv surveillance program by testing dead birds and mosquito pools for the presence of WNv and other arboviruses. Since then, we also have provided laboratory support to the state public health agencies in South Carolina and West Virginia. More than 8,500 dead birds and 106,800 mosquito pools have been tested for arboviruses, and we have isolated WNv from 2,073 dead birds and 1,690 mosquito pools. Other viruses of veterinary and/or public health concern that we have detected in these samples include Newcastle disease virus, eastern equine encephalitis virus, Cache Valley virus, and LaCrosse encephalitis virus.

SCWDS has been involved in multiple WNv research projects. In 2003, with funding from the U.S. Centers for Disease Control and Prevention, we investigated the degree to which peridomestic avian species serve as WNv amplifying hosts and sentinels in the Southeast. Funding from the Southeastern Center for Emerging Biologic Threats in 2003 enabled us to investigate avian host and vector relationships in metropolitan foci and in 2004 to investigate the eco-epidemiology of disease emergence in urban areas. We also conducted a 5-year study to identify avian

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species that are potential amplifying hosts of WNv and can serve as indicators of WNv transmission.

In studies involving rock pigeons, we assessed the extent of natural infection in free-ranging birds from metropolitan Atlanta one and two years following WNv detection in Georgia. We determined that pigeons could serve as a source of WNv for mosquitoes and found that seroprevalence rates per collection were 16%–45% in 2002 and 11%–50% in 2003. Antibodies to WNv persisted in naturally infected pigeons longer than 15 months and maternal antibodies in squabs persisted for an average of 27 days.

West Nile virus impacts on human, equine, and wild bird health are dramatically different. Human WNv cases still occur but preventive measures that reduce contact with mosquito vectors and advancements in treatment have decreased their numbers and impacts. Effective equine vaccines are available to prevent WNv infections in horses. However, wild birds may not have fared so well, and in a recent publication (George et al. Persistent impacts of West Nile virus on North American wild bird populations. 2017 PNAS 112: 14290-14294) it was reported that of 49 bird species evaluated, survival was negatively impacted in 23 (47%) species. Eleven of these species were impacted only during the initial spread of WNv, but twelve species have shown no signs of recovery. Negative population impacts for sage grouse have been of particular concern.

With WNv today, we measure human and equine disease in individual case numbers, while in wild birds we see effects in terms of major population declines. Unfortunately, as with many wildlife diseases, there are no solutions in sight. West Nile virus provides an excellent example of human, domestic animal, and wildlife health professionals uniting to fight a common foe. However, it exemplifies how varied the impacts and the possible solutions can be. (Prepared by Danny Mead and Dave Stallknecht)

Hemorrhagic Disease Just Ain't the Same

We've been working on hemorrhagic disease (HD) for six decades and one thing we can say for sure is that it gets more interesting every year. Our studies began in 1957 and initially focused on white-tailed deer, but the bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses that cause HD have a global distribution and can cause disease in sheep, cattle, and other domestic ungulates such as llamas and alpacas. Studying this disease for 60 years has allowed us to detect several changes in HD epidemiology, and some of the changes we are seeing could have significant ramifications for the health of wildlife, livestock, and human populations.

As a vector-borne disease, the distribution of HD is dependent on the presence of susceptible ruminant hosts and competent *Culicoides* vectors. Environmental conditions, such as temperature, precipitation, and other weather variables, also play a critical role. Ideal conditions can increase the abundance of vector populations and enhance the ability of EHD and BT viruses to replicate in the vector, which in turn can increase transmission potential among susceptible populations. In addition, conditions like drought may also impact disease patterns by concentrating susceptible species around dwindling water resources where the vector is abundant. Many of these potential factors remain poorly investigated.

In addition to numerous research projects, SCWDS has been monitoring HD activity nationwide for decades through two long-term datasets in order to better understand disease patterns. The first is our annual HD survey, which began in 1980 and comprises county-level reports of HD from all 50 state wildlife agencies. The second is our isolation of more than 1,200 HD viruses since 1992 from across the U.S. Collectively, these long-term data have revealed several disturbing trends. First, there has been a northern expansion of HD reporting in the U.S., primarily associated with EHD viruses. Second, there has been a steady increase in the number of exotic EHD and BT viruses detected since 2000, and at least two of them, EHDV-6 and BTV-3, appear to have become established (SCWDS BRIEFS Vol. 31, No. 3; Vol. 32, No. 3). Lastly, the scale, frequency, and intensity of HD outbreaks appear to be increasing. During 2007 and 2012, major multistate outbreaks occurred over much of the northern and Midwestern U.S. Given the multitude of ruminant hosts, *Culicoides* vectors, and viruses involved in the epidemiology of HD, the potential mechanisms driving these changes may be related to one or more host, vector, virus, or environmental factors.

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While our approach to HD always has been from the perspective of white-tailed deer health and management, the value of our ongoing surveillance and research is far reaching. Our surveillance information from deer facilitates our understanding of the disease risk in livestock, and when compiled with data from the USDA's National Veterinary Services Laboratories, it provides a comprehensive picture of where these viruses occur and the disease patterns that may be expected in the U.S. Incorporating livestock and wildlife disease information is needed to more fully understand the complex epidemiology of EHD and BT.

Similar epidemiological patterns have been reported worldwide for EHD and BT viruses. The increase we have seen in HD reports in the northern U.S., the increasing frequency of large-scale outbreaks, and the detections of exotic EHD and BT virus serotypes in the U.S. are mirrored by the northern advancement of BTV-8 and other BTV serotypes in Europe and the increased detection of EHDV-associated disease in cattle in the Middle East.

Although EHD and BT viruses do not affect humans, understanding the mechanisms behind these changing epidemiological patterns can provide insights into other vector-borne diseases, including those of humans. Many vector-borne diseases are impacted by environmental factors, such as changing land-use patterns, global warming, and other climatic, ecological, and anthropogenic factors. Some of the factors that have contributed to changes in HD patterns may drive vector species that transmit human pathogens. Although HD is well-known to many wildlife health professionals in the U.S., the changing epidemiologic patterns observed through our long-term studies demonstrate the importance of continuing and expanding these efforts into a dynamic and uncertain future. (Prepared by Mark Ruder and Dave Stallknecht)

An Island's Secret

Vesicular stomatitis New Jersey virus (VSNJV), a causative agent of vesicular stomatitis (VS) and a zoonotic pathogen, has been associated with numerous self-limiting outbreaks affecting horses and cattle in the western U.S. Outside of this historic VS area, there existed only one known focus, Ossabaw Island, a 12,000-acre barrier island on the Georgia coast, where VSNJV appeared to be endemic. The story of VSNJV on this barrier island took years of field and laboratory research by SCWDS scientists and collaborators to unravel, but it revealed an interesting example related to the complexity of today's emerging diseases and the helping hand that humans, as a species, play to enable such events.

It is uncertain how VSNJV was introduced to Ossabaw Island, but based on historical records and genetic analysis of the Ossabaw Island virus, it probably was introduced during VSNJV outbreaks in domestic livestock that occurred and subsequently disappeared in the Southeast during the 1960's. So how did it persist on Ossabaw Island? The answer to that question involves over 100 years of island history and a unique relationship between VSNJV, a sand fly vector (*Lutzomyia shannoni*), and an invasive species, the feral pig.

Swine are highly susceptible to VSNJV and can develop the characteristic vesicular lesions associated with VSV infection. On Ossabaw, we found approximately 30-60% of feral pigs seroconverted to VSNJV in any given year, and *Lutzomyia shannoni* quickly was identified as a competent virus vector. However, mysteries surrounded the potential relationship between this host and vector that appeared inconsistent with traditional arbovirus transmission and maintenance cycles. The first involved a lack of viremia in pigs during infection, and the second involved a very strange geographic distribution of VSNJV antibody-positive pigs on the island. The first mystery was solved in experimental studies at SCWDS using pigs and black flies, which are known VSNJV vectors. Our studies demonstrated that pigs could be infected by a biting vector and by direct contact with another infected pig. The infection also could be passed to uninfected flies feeding next to infected flies (co-feeding), and to flies feeding on a non-viremic animal, especially if vesicular lesions were present.

The answer to the geographic distribution question became clear after years of surveillance when SCWDS correlated forest type with the distribution of seropositive pigs. Pigs and *L. shannoni* infected with VSNJV were associated with areas of old growth maritime forest. Interestingly these areas represented the portion of the Island that was not converted to agriculture during the late 1700s and 1800s. The old growth

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forest was composed of maritime oaks that provided a reliable acorn crop for the pigs and were riddled with tree holes that provided vector breeding and resting sites. The first VSNJV infections of the year always coincided with the seasonal emergence of *L. shannoni* at these sites and once infection began, the virus was amplified by pig-to-pig transmission. Interestingly, this cycle appears to have been broken by a major reduction in the feral swine population, and from 1981 to 2008 the number of seropositive pigs and white-tailed deer on Ossabaw diminished to zero.

The mechanisms that drive emerging diseases of wildlife, humans, and domestic animals often are unclear but the Ossabaw VSNJV story provides a good example of how ecological, as well as anthropogenic factors, can combine to provide an opportunity for an introduced virus to persist in an introduced species in a very specific and localized ecosystem. (Prepared by David Stallknecht and Danny Mead)

Chagas Disease Studies

Chagas disease, which is caused by the protozoan parasite *Trypanosoma cruzi* (Tc) and transmitted by the bite of a reduvid or kissing bug (*Triatoma* spp.), is an important cause of potentially fatal cardiomyopathy and gastrointestinal disease for people in Central and South America as well as parts of North America. In southern regions of the United States it is increasingly recognized as a cause of disease in domestic dogs. The U.S. Centers for Disease Control and Prevention (CDC) has listed Chagas disease as one of five neglected parasitic infections that have been targeted for public health action.

Through funding from two NIH grants, SCWDS has investigated numerous aspects of *T. cruzi* epidemiology. We have genetically characterized parasites from numerous hosts including humans, dogs, vectors, and wildlife species (raccoons, opossums, skunks, woodrats, and other rodents). These studies found that certain strains of *T. cruzi* occurred in specific hosts, i.e., humans and opossums, had TcI, while raccoons and skunks had TcIV, and woodrats were infected with both strains. Experimental studies with raccoons and opossums confirmed the field data; animals developed chronic infections with their 'host-specific' strain.

We also evaluated novel serologic and culture-based detection methods during our experimental infection trials and expanded them to include rodent and marsupial species from South America. We found that North American strains of *T. cruzi* would infect South American species and vice versa, although the Virginia opossum was refractory to non-TcI strains of *T. cruzi*. These experimental trials also provided an opportunity to investigate alternative transmission routes and we proved that wildlife species can become infected by ingesting infected vectors; however, ingestion of infected tissues of experimentally infected animals did not result in transmission. Also, a previous infection with a North American strain resulted in decreased parasite numbers and milder lesions in the hearts of animals subsequently challenged with a virulent strain.

Although this parasite rarely causes disease in wildlife species, it is a risk for humans, dogs, and exotic animals. SCWDS worked with CDC and state public health professionals to investigate a locally-acquired human case in Louisiana, and with veterinary and public health agencies in Tennessee, Texas, and Virginia to survey dogs, determine exposure risks, or characterize parasites detected in dogs. Finally, we have worked with collaborators to study *T. cruzi* infections in capuchins in Panama, coatis in Costa Rica, and lemurs on St. Catherine's Island (Georgia).

SCWDS and collaborators at the CDC published an extensive review of *T. cruzi* and Chagas' disease in the United States in *Clinical Microbiology Reviews*, which can be accessed at: http://cmr.asm.org/content/24/4/655.long.
(Prepared by Michael Yabsley)

Deer and *E. coli* O157:H7

Cattle are considered a major reservoir of *E. coli* O157:H7 because consumption of undercooked hamburger and other beef products has been associated with many human infections, and because the bacterium has been detected in bovine feces in several countries. Human infections with this organism can result in bloody diarrhea as well as the hemolytic uremic syndrome, which can be fatal. A well-publicized, multistate outbreak in humans in 1992-93 was associated with undercooked hamburgers from a

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national fast food chain; more than 500 human cases were confirmed and four people died.

The possible role of wild deer in the epidemiology of *E. coli* O157:H7 began to receive attention as early as 1988 when a human infection was associated with undercooked venison from which *E. coli* O157:H7 was isolated. In 1995, *E. coli* O157:H7 was isolated from several persons in Oregon who had consumed improperly prepared venison jerky. Genetic analyses revealed that the bacterial isolates from the patients, jerky, and the source deer carcass were identical. *Escherichia coli* O157:H7 was cultured from deer feces collected from the ground in the Oregon area where hunters had taken the source deer; however, the genetic patterns of the fecal isolates differed from those of the outbreak isolates.

In the late 1990s, SCWDS collaborated with Drs. Mike Doyle and Tong Zhao at the UGA Center for Food Safety and conducted studies to better understand potential roles that wild white-tailed deer could play in *E. coli* O157:H7 epidemiology. We evaluated fecal shedding of *E. coli* O157:H7 in a small group of inoculated deer, determined the prevalence of the bacterium in free-ranging white-tailed deer, and investigated relationships between *E. coli* O157:H7 in wild deer and domestic cattle at the same site.

Six young, white-tailed deer orally inoculated with *E. coli* O157:H7 were shedding the organism by 1 day post inoculation (DPI). Deer continued to shed decreasing numbers of the bacteria throughout the 26-day trial, and horizontal transmission to an uninoculated deer was demonstrated. Two inoculated deer developed mild, transient, non-hemorrhagic diarrhea, but lesions of infection were not apparent in any deer despite the recovery of *E. coli* O157:H7 bacteria from the gastrointestinal tracts of deer necropsied from 4-26 DPI. Our results were similar to those of inoculation studies in bovine calves and sheep.

In field studies, we did not detect *E. coli* O157:H7 in 310 fresh deer fecal samples collected in 1997 from the ground at five wildlife management areas (WMAs). The bacterium was detected in feces collected per rectum from hunter-harvested animals from 3 of 469 (0.6%) wild deer in 1997 from the same five WMAs plus one additional site. However, enriched cultures of venison from the three positive deer failed to yield any isolates. In 1998, *E. coli* O157:H7 was not detected in 140 deer at the single positive site found in 1997; however, it was recovered from 13 of 305 (4.3%) dairy and beef cattle at the same location. Isolates of *E. coli* O157:H7 from deer and cattle at this site differed genetically. The low overall prevalence of *E. coli* O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of *E. coli* O157:H7 in the southeastern U.S. However, there may be individual locations where deer sporadically harbor the bacterium, and venison should be handled with the same precautions recommended for beef, pork, and poultry. These studies were published in *Applied and Environmental Microbiology* 67(3):1218-24. (Prepared by John Fischer)

Raccoon Roundworm: An Equal Opportunity Parasite

The raccoon roundworm, *Baylisascaris procyonis,* is a zoonotic intestinal nematode that occurs in a wide geographic range in North America. Raccoons are the primary host, but domestic dogs may harbor adult worms in their intestines and pass eggs into the environment. Migrating *B. procyonis* larvae cause severe to fatal neurologic disease in more than 150 avian and mammalian species, including humans. Approximately 50 larva migrans cases involving the eyes or brain have been reported in humans. Numbers of reported cases have increased in recent years, but this may be due to increasing awareness. Although historically regarded as rare in the southeastern U.S., reports in recent years suggest the parasite is widespread in this region. In addition, this parasite has been introduced to Europe and East Asia via raccoon translocations.

SCWDS has been involved in a variety of *B. procyonis* studies for the past 16 years, and projects have spanned the triad of public, domestic animal, and wildlife health. In addition, we have conducted active and passive surveillance for the parasite to better determine its distribution in SCWDS member states and other locations. We also have provided diagnostic support for cases, free-ranging and captive wildlife species that had *B. procyonis* larva migrans. Investigation of *B. procyonis* biology and epidemiology is critical to mitigate risk and is a great example of the One Health approach given the diverse host range, broad distribution, high prevalence in some areas, domestic “bridge” hosts (dogs), long-term persistence of eggs in the

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environment, and potential consequences of exposure.

We have worked with the Centers for Disease Control and Prevention and wildlife rehabilitation societies to investigate *Baylisascaris* exposure of wildlife rehabilitators, a group with high contact rates with raccoons. We detected *Baylisascaris* antibodies in 24 (7%) of 347 rehabilitators, and the most significant risk factors were inconsistent hand washing and practicing rehabilitation in areas where *B. procyonis* prevalence in raccoons is high.

We also conducted a survey of rehabilitators to investigate *B. procyonis*-related knowledge, attitudes, and infection control practices. We found that factors, such as education, rehabilitation experience, and membership in professional rehabilitation groups, consistently influenced knowledge score and attitudes. Among raccoon rehabilitators, their knowledge score was strongly associated with their probability of reporting correct practices. Together, these findings underscore the important role of mentorship and education among this population in influencing behavior to reduce risk for exposure. Importantly, *B. procyonis* is just one of many zoonotic pathogens rehabilitators may be exposed to while working with wild animals, and proper training is critical for risk mitigation.

Domestic dogs play an interesting role in *B. procyonis* epidemiology. They can develop larva migrans-associated clinical disease, primarily in young puppies. They also may serve as definitive hosts with patent intestinal infections, shedding eggs into the environment. We partnered with a veterinary diagnostic company to evaluate the prevalence of *Baylisascaris* eggs in pet dog feces and found that although the prevalence was low, the highest prevalences in dogs were in states with high prevalence in raccoons. However, we also detected infections in dogs in some states where *B. procyonis* is not known to occur in raccoons, likely due to a lack of surveillance. We worked with the same company to evaluate their new fecal antigen assay for detecting ascarids (*Toxocara canis*) in domestic dogs to detect *B. procyonis* infections in raccoons. To date, the assay works well, but additional validation information is being collected through experimental infections of dogs and raccoons. Our experimental infection trials also are designed to elucidate important life cycle traits in dogs, such as prepatent periods and egg output (compared to raccoons), and the possibility of larva migrans in raccoons.

This parasite has a huge host range, and clinical disease has been reported in hundreds of wildlife species, mostly in rodents, lagomorphs, and ground-dwelling birds. However, some studies have detected infections in healthy rodents, e.g., white-footed mice (*Peromyscus leucopus*). Resistance and tolerance of different host species may impact the transmission and ecology of *B. procyonis* in the wild, and we have conducted experiments to evaluate infection dynamics and survival among four *Peromyscus* species. Overall, *P. leucopus* showed a longer survival time than the other three species, despite nearly identical numbers of larvae recovered. However, the differences in partitioning of these larvae, i.e., in skeletal muscle vs. visceral organs, suggests that the ability of *P. leucopus* to "wall off" migrating larvae in viscera may slow the progression of larvae to the brain.

We developed two species-adapted serologic techniques for rodents and found that seroconversion and titer also were related to species (and therefore, survival time and tolerance of infection). These findings have an interesting connection to field surveys; two previous surveys in Illinois detected *B. procyonis* in healthy *P. leucopus* but other rodent species were not tested. We also have conducted field surveillance for *B. procyonis* in 105 free-ranging rodents of six species around Athens, Georgia, and found infections only in *P. leucopus* – leading to the question – is the lack of infections detected in healthy wild rodents due to a lack of exposure or an increased risk of clinical disease and death in these other species?

Another critical factor in *Bayslisascaris* epidemiology is the environment. We are evaluating the ability of *Baylisascaris* eggs to develop and survive in extreme temperatures, as well as methods to kill the eggs, because they are highly persistent in the environment and will survive in dilute formalin and disinfectants. Over the coming years we hope to expand these studies to continue to evaluate the risks of this parasite to people and animals and hopefully identify interventions to mitigate these risks. (Prepared by Sarah Sapp and Michael Yabsley)

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Birds, Wildlife Feeding, and *Salmonella*

Salmonella is one of the most common global causes of intestinal disease in people and animals. Despite major public education efforts and improvements to food hygiene practices, salmonellosis remains a significant source of enteric disease in the U.S., causing 1.2 million human cases and approximately 400 deaths each year. Historically, most *Salmonella*-associated illnesses were due to consumption of contaminated meat products, but there is an increasing association with produce, pet reptiles, backyard chickens, and unidentified sources. Although the incidence of human salmonellosis has remained the same during the last 20 years, there has been a shift in serovar distribution from foodborne-associated strains to environmentally-acquired strains, the source of which is not always known. Some human *Salmonella* cases have been linked to direct or indirect contact with wild birds in several independent reports, but most of the reports relied on post-outbreak surveys, which often did not investigate genetic similarities between human and avian isolates.

Although salmonellae are important human pathogens, they also can cause small to large-scale mortality events in wildlife species. Throughout the world, *Salmonella* outbreaks caused by *S.* Typhimurium have been reported in passerine birds associated with bird feeders and among nestlings of colony-nesting birds such as egrets, herons, and ibises.

In South Florida, American white ibis utilization of urban habitats has been increasing since the late 1990s, largely due to wetland loss and a constant source of food and water in urban parks in the region. Ibises now are abundant in neighborhood parks, golf courses and other artificial wetlands, where they have become sedentary and habituated to food directly provided by people, and where they regularly interact directly and indirectly with them.

Starting in 2012, SCWDS began to monitor the prevalence of *Salmonella* shedding by white ibises in South Florida to better understand the potential for people who feed ibises to become infected with *Salmonella* and to utilize ibises as environmental indicators of *Salmonella*. We found that 13% of adult/subadult ibises and 35% of nestlings were shedding *Salmonella*, and the prevalence of shedding was associated with the habitat in which they foraged: prevalence decreased as the percentage of wetlands and grasslands increased, and it increased with the proportion of developed land types, e.g., parks, lawns, and golf courses, suggesting that natural ecosystem land cover supported birds with a lower prevalence of infection.

Ibises were shedding a high diversity of *Salmonella* serovars and strain types, and 33% of the serotypes ranked in the top 20 of high significance for humans during the years of the study. Most importantly, 44% of the *Salmonella* genetic fingerprints of ibis isolates completely matched profiles in the CDC's PulseNet USA database of human cases that were not foodborne. Of these, 20% came from Florida in the same years we sampled ibises. Lastly, there was a negative relationship between the amount of wetland where ibises foraged and the number of *Salmonella* isolates from ibises that matched human cases in the PulseNet database.

We continue to monitor *Salmonell*a shedding by ibises from natural wetlands and anthropogenic environments, the serotype/strain types of isolates, and the behavior and ecology of white ibises. Our results indicate that ibises are good indicators of *Salmonella* strains circulating in their environment, and they have the potential and opportunity to transmit *Salmonella* to people. Finally, as they are a very mobile species, ibises carry *Salmonella* to natural environments where other more highly-susceptible groups (nestlings) may be detrimentally affected. These studies were published in PLoS ONE 11(10): e0164402. https://doi.org/10.1371/journal.pone.0164402.
(Prepared by Sonia M. Hernandez)

A Tale of Two Guinea Worms

The Guinea Worm Eradication Program's campaign to eradicate *Dracunculus medinensis* has led to a decrease from ~3.5 million cases in 1986 to only 25 in 2016. This parasite causes significant pain, and affected people may be debilitated for months, unable to work or attend school. Humans were the only known reservoir of the parasite, and transmission occurred via ingestion of water that contained *Dracunculus*-infected copepods (minute crustaceans) that served as intermediate hosts. However, two unusual epidemiologic factors appear to have developed: the emergence of dogs as potential reservoirs and the possible role of aquatic hosts,

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i.e., fish and/or frogs eaten raw or undercooked, in transmission to dogs and humans.

SCWDS has been working with colleagues at the Carter Center and the U.S. Centers for Disease Control and Prevention to conduct field and laboratory investigations of the natural history of this parasite and the potential role of aquatic paratenic hosts in its transmission. Paratenic hosts are not necessary for development of a parasite, but may serve to maintain its life cycle. Although we are conducting work in Chad, Africa with *D. medinensis*, some experiments are logistically challenging to conduct there, so we are using a raccoon local parasite, *D. insignis*, as a model system.

Several experimental studies have shown that *D. medinensis* and *D. insignis* can utilize a diversity of amphibian species as paratenic hosts. Furthermore, we found that third stage larvae can remain in amphibians through metamorphosis and be present as infective third-stage larvae in adult frogs for up to eight months. In contrast, we have failed to establish *Dracunculus* infections in numerous fish species, although it is possible that some species of catfish may be suitable paratenic hosts. Fish appeared generally refractory to infection with *D. medinensis* and *D. insignis*, but we did show that they can act as short-term transport hosts. Transmission occurred when fish ingested *Dracunculus*-infected copepods and were consumed by an appropriate definitive host (we used domestic ferrets). This has implications for the field, and campaigns have been implemented asking fisherman and others to bury or burn the guts of cleaned fish. Although people may not consume this material, it often was available for dogs to ingest.

We are undertaking trials to assess the likelihood of dogs getting infected via the traditional transmission route, i.e., ingestion of *Dracunculus*-infected copepods, as well as by ingestion of infected frogs. Additionally, we are conducting an experimental study to evaluate the efficacy of a compound to prevent or treat canine infections with *Dracunculus*, because it would greatly aid eradication efforts if treatment or prevention of dog infections were possible.

We have been examining possible definitive and paratenic hosts for *D. insignis* in an effort to better understand host-specificity and transmission. Similar to previous studies, we found that raccoons are frequent hosts, but we also found that opossums at our field study site commonly were infected. In addition, we have a particular interest in North American river otters, which are known hosts of *D. insignis* and *D. lutrae* in Canada. Using a combination of morphologic and molecular data, we identified two previously undescribed *Dracunculus* species in river otters from Florida and North Carolina.

Collectively, our data show that the diversity of *Dracunculus* spp. is higher than previously recognized, and we have elucidated several novel methods in which this parasite can be maintained or transmitted in nature. We regularly present our findings to the Carter Center and the Guinea Worm Eradication Program, and they immediately use these data to adapt their intervention efforts. We publish our data in open access journals to ensure ready access for all parties involved in the eradication campaign and three articles can be found in *Emerging Infectious Diseases* (https://wwwnc.cdc.gov/eid). (Prepared by Chris Cleveland and Michael Yabsley)

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